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Laboratory Diagnosis of Meningitis

S. Nagarathna, H. B. Veenakumari and A. Chandramuki
Department of Neuromicrobiology,
National Institute of Mental Health and Neurosciences (NIMHANS),
Bengaluru Karnataka,
India

1. Introduction

Meningitis is an infection of the membranes (meninges) surrounding the brain and spinal cord. Meningitis is usually of multiple etiology-bacterial, fungal or viral yet bacteria remain the common etiological agent (Reid & Fallon, 1992). Meningitis can be acute, with a quick onset of symptoms, or chronic, lasting a month or more, or can be mild or aseptic, but the emphasis should be on identification of cause so that appropriate interventions can be applied.

Bacterial meningitis continues to be a potentially life threatening emergency with significant morbidity and mortality throughout the world and is an even more significant problem in many other areas of the world, especially in developing countries (Carbonnelle, 2009, Brouwer et al., 2010).

Types of bacteria that cause bacterial meningitis vary by age group. Currently, the average age of contracting meningitis is above 25 years with Streptococcus pneumoniae, Neisseria meningitidis and Haemophilus influenzae being the most common pathogens (Ogunlesi et al., 2005, Brain, 2004 as cited in Maleeha Aslam et al., 2006). Trauma to the skull gives bacteria the potential to enter the meningeal space. Similarly, individuals with a cerebral shunt or related device are at increased risk of infection through those devices. In these cases, infections with Staphylococci, Pseudomonas aeruginosa and other gram-negative bacilli are more likely. Recurrent bacterial meningitis may be caused by persisting anatomical defects, either congenital or acquired, or by disorders of the immune system. (Brouwer et al., 2010)

Tuberculous meningitis (TBM), is common in those from countries where tuberculosis is common, and is also encountered in those with immune problems, such as AIDS.

Despite advancement in vaccine development and chemoprophylaxis bacterial meningitis remains a major cause of death and neurological disabilities which can be prevented by rapid and accurate diagnosis with prompt treatment which is essential for good outcome (Carbonnelle, 2009).

Viral meningitis is generally less severe and clears up without specific treatment. Viral ("aseptic") meningitis is serious but rarely fatal in people with normal immune systems. Usually, the symptoms last from 7 to 10 days and the patient recovers completely. Often, in

early phases of viral meningitis and bacterial meningitis, the symptoms are almost similar (Carbonnelle, 2009).

Fungal meningitis is rare, but can be life threatening. Although anyone can get fungal meningitis, people at higher risk are those who have AIDS, leukemia, or other forms of immunodeficiency. The most common cause of fungal meningitis in HIV, is Cryptococcus spp. In the last two decades, more elaborative use of intensive care units for serious medical disorders, advancements in transplant procedures and concomitant use of immunosuppressive therapies as well as the pandemic spread of HIV, etc. have increased the incidence of Central Nervous System (CNS) fungal infections which present with various clinical syndromes: meningitis commonly. The clinical picture may mimic TBM and therefore, needs careful evaluation. The CNS mycoses carry higher risks of morbidity and mortality as compared to other infective processes and therefore promptly require precise diagnosis and appropriate medical and/or surgical management strategies to optimize the outcome (Raman Sharma, 2010).

Chemical meningitis can develop after neurosurgical procedures and can be differentiated from bacterial meningitis by Cerebrospinal fluid (CSF) glucose levels and CSF White Blood Cell (WBC) values.

The causes of non–infectious meningitis include cancers, systemic lupus erythematosus, drug induced, head trauma, brain surgery etc.

2. Collection, transportation, receipt and storage of CSF

Direct testing of CSF is the most accurate way to confirm the diagnosis of bacterial meningitis. CSF should be collected from all the cases with suspected meningitis before commencement of antimicrobial therapy, unless lumbar puncture (LP) is contraindicated.

Petechial fluid can be another specimen in cases with meningococcal meningitis. Petechial lesions, if present, may be gently irrigated by injecting 0.2 ml of sterile saline solution using a small syringe with a fine needle and the fluid collected for smear and culture.

Early diagnosis is essential and is best established by laboratory examination of CSF. However, therapy should not be dependent or delayed pending lumbar puncture or laboratory results (WHO).

To initiate the definitive identification of a bacterium responsible for meningitis, CSF specimens should be obtained from patients with clinical signs and symptoms of meningitis and should be transported to the laboratory without delay. N.meningitidis, S.pneumoniae, and H.influenzae are fastidious organisms that may not survive long transit times.

The processing of a CSF specimen is one of the few clinical microbiology procedures that must be done immediately. Laboratorians should always record the date and time a specimen was received. Usually, three or more tubes of CSF are collected during a LP procedure.

The tubes should be numbered in sequential order with tube number one containing the first sample of CSF obtained. The CSF in tubes 1, 2, and 3 most often are examined for chemistry, microbiology, and cytology, respectively (Gray & Fedorko, 1992). However, the particular tests performed on tubes 2 and 3 are subjective and probably best determined by the laboratarians.

Contamination with skin flora and disinfectant will be ruled out after the first tube of CSF is collected. The probabilities of detecting microorganisms by staining and by culturing are related to the volume of specimen that is concentrated and examined (Tenney et al., 1982 as cited in Gray & Fedorko, 1992)

CSF volumes of 2 to 3 ml are usually sufficient to detect bacteria, but for mycological and mycobacterial investigations a minimum of 5 ml (preferably 10 to 15 ml) of CSF is required. If only a small amount of CSF is received with requests for multiple assays, the order of priority of the tests is determined after discussion with the physician.

The specimen should not be refrigerated before subjecting to microbiological tests as it may prevent the recovery of the organisms; fastidious organisms may not survive variations in temperature (Kasten, 1990 cited in Gray & Fedorko, 1992)

CSF specimens should be stored at room temperature or at 37^{o}C if they cannot be processed immediately or till microscopy and bacterial cultures are performed, after which it can be refrigerated for further use (WHO).

3. Laboratory diagnosis of bacterial meningitis

Bacterial meningitis is a significant cause of mortality and morbidity worldwide. Neurological outcome and survival depend largely on damage to CNS prior to effective antibacterial treatment. Quick diagnosis and effective treatment is the key to success. The diagnostic dilemma in acute pyogenic meningitis is due to large spectrum of signs and symptoms.

3.1 Examination of CSF

The CSF should arrive still warm and either be examined immediately or placed in an incubator for examination within an hour. If delay is anticipated either in transportation to the laboratory or for examination, CSF should be divided into two containers: one in a plain bottle and the other in a bottle having a few drops of glucose broth. In the laboratory, CSF from the plain bottle can be used for making smears for staining whereas cultures are done from containers having CSF in glucose broth. The residual CSF should be preserved frozen in the CSF bank for further assessment and evaluation with evolving /additional contributory findings.

An examination of CSF involves the following:

3.1.1. Macroscopic examination.
3.1.2. Cytological examination.
3.1.3. Examination of Gram stained smear.
3.1.4. Culture and antimicrobial susceptibility testing.
3.1.5. Latex agglutination test for antigen detection.
3.1.6. Other diagnostic methods.

3.1.1 Macroscopic examination

By appearance, the CSF is normally clear like water; cloudy, purulent, bloody or pigmented CSF as per the disease states.

Hazy, cloudy, turbid CSF indicates either metastatic spread of tumors into the CNS or pleocytosis or severe meningeal infection; Opalescent CSF may be suggestive of cryptococcal meningitis. The turbid nature of the CSF is attributable to both the bacteria and leukocytes present.

Hemorrhagic CSF may be indicative of Anthrax meningitis with supportive clinical findings.

Frank clots or pellicles in CSF occur only if protein concentration exceeds 15g/L.

Xanthochromia of CSF is seen within 4 weeks of a cerebral hemorrhage.

In evaluating patients with suspected meningitis or encephalitis, a careful history along with biochemical and cellular analysis of CSF is required.

3.1.1.1 CSF glucose

CSF glucose concentrations <45 mg/dL are indicative of bacterial meningitis. (Bonadio, 1992) CSF glucose concentrations depend on serum concentrations and should always be tested on paired samples. A CSF/serum ratio cut-off of <0.4 is helpful in distinguishing between bacterial and aseptic meningitis with a sensitivity and specificity of 91% and 96%, respectively. (Genton & Berger 1990)

The individual predictors of bacterial meningitis consists of a glucose concentration of less than <40 mg/dl and a ratio of CSF to blood glucose of <23 mg/dl (Brouwer et al., 2010, Gray & Fedorko, 1992).

Chemical meningitis can be differentiated from bacterial meningitis by CSF glucose levels (< 10 mg/dL) and CSF WBC values (>7500 cells/µL) (Forgacs et al., 2001).

3.1.1.2 CSF protein

Despite typical CSF findings, the spectrum of CSF values in bacterial meningitis is so wide that the absence of one of more of the typical findings may not affect the diagnosis. In community-acquired bacterial meningitis, (CABM) only 50 percent may have a CSF glucose above 40 mg/dL (2.2 mmol/L), less than half cases may have a CSF protein below 200 mg/dL, CSF protein measurements of >55 mg/dL are diagnostic of bacterial, fungal and tubercular meningitis (Bonadio, 1992)

3.1.2 Cytological examination

In untreated bacterial meningitis, the WBC count is elevated, usually in the range of 1000–5000 cells/mm3, although this range can be quite broad (<100 to >10, 000 cells/mm3).

Bacterial meningitis usually leads to a neutrophil predominance in CSF, typically between 80% and 95%; 10% of patients with acute bacterial meningitis present with a lymphocyte predominance (defined as >50% lymphocytes or monocytes) in CSF (Tunkel et al., 2004). Preponderance of CSF polymorphonuclear cells may be used to distinguish bacterial meningitis from other causes. It is important to note that a false-positive elevation of the CSF WBC can be found after traumatic lumbar puncture, or in patients with intracerebral or subarachnoid hemorrhage in which both red blood cells and white blood cells are introduced into the subarachnoid space. In these instances, the following formula can be used as a correction factor for the true WBC count in the presence of CSF red blood cells (RBC).

$$\text{True WBC in CSF} = \text{Actual WBC in CSF} - \frac{\text{WBC in blood x RBC in CSF}}{\text{RBC in blood}}$$

Generalized seizures may also induce a transient CSF pleocytosis (primarily neutrophilic), although the CSF WBC count should not exceed 80/microL in this setting. However, CSF pleocytosis should not be ascribed to seizure activity alone unless the fluid is clear and colorless, the opening pressure and CSF glucose are normal, the CSF Gram stain is negative, and the patient has no clinical evidence of bacterial meningitis.

Since the CSF is hypotonic neutrophils may lyse, and counts may decrease by 32% after 1 hour and by 50% after 2 hours in CSF specimens, held at room temperature (Steele et al, 1986, as cited in Gray & Fedorko., 1992), hence a delay may produce a cell count that does not reflect the clinical situation of the patient.

Characteristic CSF findings for bacterial meningitis consist of polymorphonuclear pleocytosis, hypoglycorrhachia, and raised CSF protein levels (Van De Beek et al., 2006, as cited in Brouwer et al., 2010). However, low CSF WBC do occur, especially in patients with septic shock and systemic complications (Heckenberg et al, 2008 and Weisfelt, et al., 2006 as cited in Brouwer et al., 2010). A relationship between a large bacterial CSF load, lack of leukocytes response (Tauber, et al., 1992 as cited in Brouwer et al 2010.**,)**, probably indicating excessive bacterial growth and poor cell response, is well known especially in cases of pneumococcal meningitis. (Brouwer et al., 2010)

In CABM, 10-15 percent have a CSF WBC below 100/microL (Durand et al 1993). In some proportion of the patients of CABM, around 15 percent may not exhibit characteristic CSF findings (Van de Beek et al 2004).

Some patients have milder CSF abnormalities, which cannot usually be identified. Potential causes include early presentation, recent prior antibiotic therapy etc

WBC differential may be misleading early in the course of meningitis, as in small proportion there may be an initial lymphocytic predominance and viral meningitis may initially be dominated by neutrophils. (Arevalo, 1989, as cited in Seehusen, 2003).

In our study, the cell counts of the CSF samples ranged from acellular to sheets of cells, not countable on the hemocytometer. A predominance of polymorphonuclear cells was the common feature in all cases with high cell counts.

In some facilities, clinical and management decisions are made on the cell type and the number. Thus, patients with cerebrospinal fluid pleocytosis, on further assessment, may have a preponderance of polymorphonuclear cells that would prompt a diagnosis of bacterial meningitis. 14 cases in our study had a CSF cell count of < 100 cells/cumm, 2 of which had a cell count of 10 cells/cmm and one had no cells.All these cases yielded S.pneumoniae on culture. Normal or marginally elevated CSF white cell counts are known to occur in 5-10% patients and are associated with an adverse outcome. (Van De Beek et al., 2004, as cited in Mani et al 2007)

3.1.3 Examination of Gram stained smear

It is preferable to make a smear from CSF at the time of collection itself, for direct demonstration of organisms.

The Gram stained smear made either directly from the CSF or from the centrifuged deposit can reveal not only the Gram character of the causative organism, but can also clinch the diagnosis in some cases. Gram stain may not be interpretable in grossly blood-stained samples.

Although Gram staining of CSF sediment is a very useful, cheap and fairly rapid method of identification of organism, the sensitivity in developing countries is only 25-40% (Singh, 1988, as cited in Sanya, 2007**)** when compared to 80-85% in developed countries (Gray & Fedorko, 1992).

CSF Gram staining may swiftly identify the causative microorganism for patients with suspected bacterial meningitis. The additional value of Gram staining for CSF culture negative patients is elucidative (Brouwer et al 2010). In 1/3rd of the cases with bacterial meningitis defined by CSF parameters may have negative CSF cultures; around 50% of the CSF culture negative patients have a positive Gram stain with equal percent of patients being pretreated with antibiotics (Bryan, 1990, as cited in Brouwer et al., 2010).

The Gram stain is positive in 10 to 15 percent of patients who have bacterial meningitis but with negative CSF cultures (Durand 1993).

In developing countries among suspected meningitis cases, CSF Gram staining can identify the causative organisms in 2/3rd, and CSF culture is positive in 1/10th of the pretreated patients (Shameem et al., 2008 as cited in Brouwer et al, 2010).

Gram staining correctly identifies the pathogen in 69 to 93% of patients with pneumococcal meningitis.

The reported yield for meningococcal meningitis is highly variable and may range from 30-89%, in a spectrum of all-ages, paediatric and untreated adult patients; (Brouwer et al., 2010)

The reported sensitivities of CSF Gram staining vary considerably for different microorganisms. CSF Gram staining correctly identifies the organism in 50 to 65% of children and in 25 to 33% in adults with H. influenzae meningitis (Brouwer et al.,).

The reported sensitivity of Gram stain for bacterial meningitis has varied from 60 to 90 percent; however, the specificity approaches 100 percent (van de Beek et al., 2004, Fitch &Van de Beek 2007). In CABM, CSF Gram stain had a sensitivity of 80 percent and specificity of 97 percent (Van de Beek 2004).The yield of both Gram stain and culture may be reduced by prior antibiotic therapy.

In our study, Gram stain provided an evidence of the causative bacteria in 253 (65.7%) patients. Our relatively high yield of pathogens on Gram stain can be attributed to the routine use of cytospin to concentrate the smear. (Mani et al 2007). The chances of recovery of bacteria in CSF Grams up to 100-fold, can be intensified by replacing conventional centrifugation with cytospin centrifugation. (Shanholtzer, et al 1982., as cited in Gray & Fedorko., 1992,). This increase is comparable to the concentration of 100 ml of CSF to a volume of 1.0 ml by conventional centrifugation. Cytospin-prepared smears not only increased the positivity of the smears, the morphology of the cells were well preserved with uniform distribution of the cells. This is especially helpful in partially treated pyogenic meningitis, which can mimic TBM posing a diagnostic dilemma for clinicians.

It is to be noted that the preliminary report of Gram staining should be conveyed immediately on the basis of initial observations.

3.1.4 Culture and antimicrobial susceptibility testing

Culture is the gold standard for determining the causative organism in meningitis. Culture of CSF is also infrequently performed in many health institutions in developing countries.

After the receipt, specimen should be cultured at the earliest. CSF should also be inoculated into enrichment medium like sodium thioglycollate broth along with solid media like enriched, selective or differential media. Incubate in air plus 5-10% carbon dioxide.

Aerobic CSF culturing techniques are obligatory for CABM. Anaerobic culture may be important for postneurosurgical or posttraumatic meningitis or for the investigation of CSF shunt meningitis.

Sensitivity of CSF culture does not exceed 40% with results available only after 2-3 days (Singh, 1988 and Gray & Fedorko, 1992 as cited in Sanya et al., 2007) While CSF culture are only positive in about 80% of the time(Coyle, 1999, Zunt, & Marra 1999), negative or inconclusive culture results may be seen in patients with partially-treated meningitis and those with atypical bacteria, and Mycobacterium tuberculosis.

In a series of 875 meningitis patients for whom the diagnosis was defined by a CSF WBC count of over 1, 000 cells per mm3 and/or more than 80% polymorphonuclear cells, the CSF culture was positive for 85% of cases in the absence of prior antibiotic treatment. CSF cultures were positive for 96% of patients if meningitis was due to H.influenzae, 87% of patients with pneumococcal meningitis, and 80% of patients with meningococcal meningitis (Bohr, et al., 1983 as cited in Brouwer et al., 2010)

The yield of CSF culture is lower for patients who have been pretreated before lumbar puncture. Pretreatment for more than 24 h is associated with a further decrease of positive CSF cultures.Two large case series reported decreases in yield from 66 to 62% and 88 to 70% if patients were pretreated with antibiotics (Bohr, et al., 1983, Nigrovic, et al., 2008 as cited in Brouwer et al 2010,.).

A decrease in culture positivity can be expected for pretreated patients with clinically defined meningitis. Meningococcal meningitis diagnosed either by culture or by PCR can show positive CSF cultures though in low percentage of patients receiving pretreatment and 4-5 times more for those who did not receive any treatment (Bronska, 2006 as cited in Brouwer et al., 2010). In our study, 40.8% of culture positivity was noted (Mani et al., 2007) and 10% in a similar study (Shameem et al., 2008 as cited in Brouwer et al., 2010).

For patient management, physicians hardly wait for antimicrobial susceptibility reports, as the treatment is usually initiated with more than one antimicrobial agent. Nevertheless, the laboratory must have its policy regarding testing and reporting for antimicrobial susceptibility. For organisms such as N. meningitidis and beta-haemolytic streptococci with predictable susceptibility patterns, there is no need of performing antimicrobial susceptibility testing. For all other organisms antimicrobial susceptibility tests have to be performed, as per standard methodology or automated systems according to accessibility and affordability to enhance the turnaround time.

In a world of increasing resistance to antibiotics and emerging pathogens, culture combined with susceptibility testing remains the gold standard for diagnosis (Brouwer et al., 2010).

3.1.5 Latex Agglutination Test (LAT) for antigen detection

Tests are available to detect antigen in the CSF which are feasible at peripheral as well as intermediate laboratories where fundamental, preliminary facilities are not available.

Of all these tests latex agglutination is a tool for screening and is rapid, sensitive, and specific less labour-intensive, are highly sensitive and specific much more expensive than routine culture and are not available for routine use in developing countries(Sanya et al., 2007).

Kits are commercially available containing reagents for detecting antigens and the test can be performed under field conditions.

LAT detects the antigens of the common meningeal pathogens in the CSF, although these tests are not routinely recommended.

LAT is a diagnostic test that has been utilized for providing speedy results.These tests utilize serum containing bacterial antibodies or commercially available antisera directed against the capsular polysaccharides of meningeal pathogens (Tunkel et al., 2004). Agglutination with the respective latex reagents indicates presence of corresponding antigen in CSF and is diagnostic.

Antigen testing may result in few indeterminate, false negatives, and false-positives. True-positive results do not appear to modify the decision to administer antimicrobial therapy, therefore reserved for specific clinical circumstances. The suggested indications are-

- Initial negative CSF Gram staining and CSF cultures;
- Partially-treated and pretreated patients with negative CSF culture (Shivaprakash et al., 2004)

The false-negative LAT could be possibly because of low antigen titres in the CSF. It is possible that the antiserum in diagnostic LAT kits does not detect all the capsular serotypes prevalent in the particular geographical area or probably as yet unrecognized serotypes are the causative agents in such cases.

The reported sensitivities of LAT of CSF samples from patients with bacterial meningitis ranged from 78 to 100% for H. influenzae type b meningitis, 59 to 100% for pneumococcal meningitis, and 22 to 93% for meningococcal meningitis (Brouwer et al 2010.,).

In negative CSF cultures with clinical presentation and CSF parameters compatible with bacterial meningitis, CSF latex agglutination has a lower sensitivity for detecting bacteria (Tarafdar et al 2001 as cited in Brouwer et al 2010,.).

In CSF specimens positive for the causative microorganism, the positivity of the LAT can be upto 100% (Perkins, etal., 1995 as cited in Brouwer et al., 2010).

A strong decline in the sensitivity of LAT is expected among patients with antibiotic pretreatment prior to lumbar puncture (Bronska, et al., 2006, as cited in Brouwer et al 2010.,). The additional value of LAT is therefore limited.

In our study, the LAT was positive in 54.6% of cases. In the culture negative cases, LAT was positive in 49.6%, wherein 82% of these positive cases were also positive by Gram's stain, but 18% samples which did not show any evidence of the pathogen on either Gram's stain or culture, were positive by LAT, thus helping clinch the diagnosis.

A negative test does not rule out pyogenic meningitis and should be considered as an adjunct test and needs to be interpreted cautiously, considering the patient clinical condition and several other factors into consideration.

3.1.6 Other laboratory tests

3.1.6.1 Blood cultures

Blood cultures can be useful in a situation where CSF cannot be obtained before the administration of antimicrobials. Blood cultures can enhance the identification of the causative organism which follow haematogenous route to reach the meninges (WHO).

Blood cultures are often positive and valuable to detect the causative organism and establish susceptibility patterns if CSF cultures are negative. Majority of the patients with bacterial meningitis have positive blood cultures.

Blood culture positivity differs for each causative organism:

- 50 to 90% of H. influenzae meningitis
- 75% of pneumococcal meningitis and
- 40% of children and 60% of adult patients with meningococcal meningitis

Extended culture is preferred and the inoculated media should not be discarded before seven days of incubation.

Cultures obtained after antimicrobial therapy are much less likely to be positive, the yield will be decreased by 20% for pretreated (Brouwer et al 2010.,) and could be even higher in developing countries.where the patients are partially treated either by the local practitioners or due to availability of drugs over the counter because of not-so stringent policies, before they approach the referral centres.

The automated blood culture systems for example BACTEC system available in less-developed countries, has time-to-detection advantages with reasonable turnaround time, and has to be compared with standard culture techniques available at most health facilities in such countries. Therefore, culture results might be overly sensitive. Hence excluding cultures of presumed contamination and also to note what proportion of the cultures are contaminated, is of primary importance.

3.1.6.2 Polymerase Chain Reaction (PCR)

Diagnosis of bacterial meningitis has long been based on classical methods of Gram stain, culture of CSF, and serological tests. The performance of these methods, especially culture and direct smear, is thwarted by failure to detect bacteria in pretreated cases and reluctance to perform lumbar punctures at admission. Indeed, patients with meningitis frequently receive antibiotics orally or by injection before the diagnosis is suspected or established. Thus an alternative method has become necessary to help clinicians and epidemiologists for the management and control of bacterial meningitis. Nucleic acid amplification tests such as

PCR assays are highly sensitive and specific and have been evaluated for their effectiveness in detecting the presence of bacterial DNA in CSF from patients with suspected and proven bacterial meningitis.

PCR-based assays are useful adjuncts to conventional bacterial culture and antigen detection methods in establishing the bacterial etiology in meningitis in settings where substantial numbers of specimens are culture negative.

With the advent of quantitative PCR methods, monitoring prognosis of patients on therapy is possible with a greater degree of accuracy. The high sensitivities of PCR protocols ensure detection even with a minimal amount of the DNA or RNA present.

PCR may play a greater role in the diagnosis of bacterial meningitis. It has been suggested that PCR is useful in establishing the diagnosis of viral meningitis in CSF culture negative patients (Kaplan, 1999).

A study including culture-confirmed CABM evaluated the diagnostic accuracy of a broad-range PCR for H. influenzae, S. pneumoniae, and N. meningitidis had an overall specificity of 100 percent The sensitivity for H. influenzae, S. pneumoniae and N.meningitides, was 92%, 100%, and 88%; respectively (Corless et al., 2001 as cited in Brouwer et al 2010.,).

By multiplex assay, it is found that in bacterial meningitis patients defined by positive CSF culture, positive CSF Gram stain, and based on clinical suspicion with negative cultures, PCR has high sensitivities for H. influenzae, S. pneumoniae, and N. meningitidis with a specificity of 100% for all three microorganisms (Tzanakaki et al 2005., as cited in Brouwer et al.,).

In an another, study the sensitivities and specificities of multiplex PCR for CSF diagnosed by CSF culture, latex agglutination test, PCR, or Gram stain may be comparatively lower for H. influenzae, S. pneumoniae, and, N. meningitides.

The incremental value of PCR next to culture, Gram stain, and latex agglutination is high.(Parent du Châtelet et al 2005 as cited in Brouwer et al.,).

Meningococcal DNA detection by PCR has been used widely and is performed routinely for patients with suspected meningococcal meningitis and negative CSF cultures in many parts of the world (Welinder-Ol et al., 2007 as cited in Brouwer et al.,). In the United Kingdom, a large proportion of meningococcal disease cases are now diagnosed by PCR without culture (Gray et al., 2006 as cited in Brouwer et al.**,)** PCR detection of meningococcal DNA requires special techniques and is expensive.

Pretreatment with antibiotics may decrease the sensitivity of PCR of CSF samples compared to untreated patients (Bronska et al 2006., as cited in Brouwer et al.,)**.** PCR can also be a useful tool for the swift typing of meningococcal strains in an evolving epidemic.

A high bacterial load determined by quantitative PCR has been associated with unfavorable outcomes of both pneumococcal and meningococcal disease (Brouwer et al 2010.).

Data on PCR detection of group B streptococci in CSF are limited, and group B streptococci have been tested only with multiplex PCR detection assays (Chiba et al 2009 as cited in Brouwer et al.,).

An initial study of the PCR detection of L. monocytogenes in patients with bacterial meningitis showed that a high concentration of bacteria in the CSF is needed for PCR

detection. Recent studies of multiplex PCRs including L. monocytogenes showed lower detection thresholds. The sensitivity, specificity, and incremental value of PCR in L. monocytogenes meningitis are unclear, as only one patient was included in each of these studies (Brouwer et al., 2010).

Problems with false-positive results have been reported. In our study, PCR assay showed an overall sensitivity of 100% by positively identifying all the culture, smear and antigen positive cases. Additional cases, which could not be diagnosed by the conventional techniques, were also picked by this assay. Out of the 32 controls, the assay showed a positive result in only two cases of autopsy proven TBM cases, suggesting the possibility of a mixed underlying infection in those particular patients (Mani et al., 2007).With high prevalence of tuberculous infection in developing countries and the impaired immune status of patients, co- existent pneumococcal and tubercular meningitis may not be so uncommon (Garg et al., 2008). However, further refinements may make PCR a more useful tool in the diagnosis of bacterial meningitis in a clinical setting especially when results of CSF Gram stain and culture are negative.

3.1.6.3 Flow cytometry

Recently, flow cytometry with a dedicated bacterial channel has (Sysmex UF-1000i) possible application in automated cell counting and this novel approach in the differential diagnosis of meningitis has been explored.(Nanos, and Delanghe 2008.)

3.1.6.4 Inflammatory markers

Diagnosis and management of bacterial meningitis require various biological tests and a multidisciplinary approach.

Occasionally, there is difficulty in distinguishing bacterial meningitis from aseptic meningitis on the basis of commonly used laboratory tests. A number of recent studies strongly suggest that measurements of C- reactive protein (CRP) in CSF could reliably discriminate between them (Singh 1994). Its advantages include its very low serum levels in normal individuals, a rapid rise within 12 to 24 hours of infection and a large incremental increase thereafter. Qualitative assay of CRP is simple to perform at the bedside by medical staff. Sound clinical judgement combined with qualitative CRP assay as an adjunctive test should provide a rational basis for treatment decision in the management of bacterial meningitis. This will significantly reduce unnecessary antimicrobial therapy, ensure adequate dosage of antibiotics and will also prevent emergence of resistant strain of microorganisms (Col PL Prasad et al., 2005**)** especially in those situations where facilities for performing bacterial cultures of antibiotic susceptibility testing are not available (Singh 1994).

Several other analytes are reported to be of significance in the diagnosis of meningitis including adenosine deaminase, cytokines, and lactate concentrations although their diagnostic significance has yet to be established. CSF β_2-microglobulin and neopterin concentrations are used in the diagnosis of AIDS dementia complex with some success.(McArthur, et al., 1992, Watson & Scott 1995).

The limited diagnostic utility of most CSF biochemical analytes is attributable to several causes including timing of sample acquisition and the need for serial determinations of analytes. Most of these markers lack neurospecificity and in patients with a damaged blood-

brain barrier, diagnostic values of intrathecal concentrations are questionable. Organism-specific antibody index evaluation is useful in determining whether the specific viral or bacterial antibody under evaluation is being synthesized intrathecally and is of tremendous diagnostic significance. (Reiber and Lange 1991). Measurements of all biochemical analytes in the CSF should be reviewed in conjunction with the albumin index; an elevated albumin index is evidence of a compromised blood-brain barrier and leakage from the serum into the CSF. Furthermore, contamination by erythrocytes from a traumatic spinal tap or intracerebral hemorrhage will cause variations in measurements; therefore, an isolated determination of CSF analytes can be an adjunctive tool for the differential diagnosis of unknown CNS disease (Thompson 1995).

Patients with listerial meningitis often do not have characteristic CSF findings, with relatively low CSF leukocyte counts and high CSF protein concentrations (Brouwer, et al., 2006 as cited in Brouwer et al., 2010). A mononuclear cell predominance in the CSF is found more frequently than for other types of bacterial meningitis (Clauss, and Lorber 2008 as cited in Brouwer etal., 2010).Listeria demonstrates "tumbling motility" in wet mounts of CSF. The yield of Gram staining for Listeria meningitis is low, ranging from 23 to 36% for both children and adults, CSF Gram stain that show diphtheroids should prompt heightened awareness for the possibility of Listeria infection, particularly in immunocompromised patient. The sensitivity, specificity, and incremental value of PCR in L. monocytogenes meningitis are unclear, as only few cases have been studied (Brouwer et al 2010).

Nocardial meningitis results in findings typical of bacterial meningitis. Since nocardiae grow slower than common bacteria, the microbiology laboratory should always be notified when nocardiosis is clinically suspected.

Meningitis caused by anaerobic bacteria is rare. It is advisable to perform anaerobic culture on cerebrospinal fluid from patients with identifiable risk factors such as when sinus, otitic or mastoid symptoms precede or accompany the onset of meningitis in children or adults. The presence of irregularly stained gram negative rods in the CSF or meningitis unresponsive to empiric antibiotics should also raise the suspicion of anaerobic infection (sree neelima et al., 2004).

CSF analysis has been reported to be normal for 20% of patients with culture-proven nosocomial meningitis (Weisfeltet al., 2007).

In case of anthrax meningitis, the CSF is grossly hemorrhagic with few PMN neutrophils and numerous gram-positive bacilli.

In patients with purulent CSF indices with no evidence of bacteria on Gram smear it is very important to examine a wet mount of CSF for amebic trophozoites. Molecular methods have also been developed for the rapid identification of meningoencephalitis caused by Naegleria.

Adults with S. agalactiae meningitis have typical CSF findings(Domingo, 1997and Dunne and Quagliarello, 1993, as cited in Brouwer et al 2010).CSF WBC counts are inconclusive for many neonates with meningitis due to S. agalactiae with a normal CSF examination in a very small proportion of patients (Ginsberg, 2004, as cited in Brouwer et al, 2010).

Lyme disease is caused by Burkohlderia burgdorferi (B. burgdorferi) and is known to be neurotropic. The results of laboratory testing among patients with neurologic Lyme disease vary depending on the stage of the illness. In very early CNS involvement (meningismus) or

late-stage infection (encephalopathy), the CSF may appear normal.When clinical signs of meningitis or encephalitis are present, CSF may reveal a mononuclear pleocytosis, mildly increased protein, and, in some cases, an elevated IgG index or oligoclonal immunoglobulins. Intrathecal anti- B. burgdorferi antibody production is present in 70%-90% of patients with Lyme meningitis (Coyle 1992 as cited in Brian and Jenifer1994). Given the limitations of diagnostic tests, clinicians need to consider clinical factors that would aid in the diagnosis of Lyme disease. These include a history of an erythema migrans rash or Ixodes tick bite, exposure to a Lyme endemic area, and the combination of neuropsychiatric and extraneural symptoms as it is a multisystemic illness. Once in the CNS, B. burgdorferi, like T. pallidum, may remain latent, only to cause illness months to years later. (Logigian 1990 as cited in Brian and Jenifer 1994).

4. Important points in diagnosis

Acute bacterial meningitis is a life threatening condition and specimen deserves immediate processing and diagnosis is best established by laboratory examination of CSF.

If there is an insufficient volume of sample for carrying out all the investigations, prioritize the tests as per the medical advice.

The CSF should be examined fresh at the earliest, as on storage cells disintegrate and produce a cell count that does not reflect the true clinical situation.

Cell count to be performed on the uncentrifuged CSF specimen.

When a clot is present in CSF, it invalidates the CSF cell count.

Results of cell count and type, Gram stain, antigen detection assays etc. and positive cultures must be conveyed to the physician caring for the patient (if possible) as soon as the results are available, and a permanent record of the communication and the name of the person who was notified of the initial results should be made.(WHO)

The management of the patient should be based on immediate cell-count and Gram stain smear results.

Notify to the public health authorities on isolation/detection of any epidemic prone pathogens (e.g. N.meningitidis).

5. Tuberculous meningitis (TBM)

The non-specific clinical and cerebrospinal fluid (CSF) features have made TBM often difficult to diagnose with certainty, especially at early stages and has to be differentiated from a plethora of other infectious and non-infectious meningitis such as viral, bacterial cryptococcal or carcinomatous meningitis, often resulting in diagnostic dilemma.TBM is usually diagnosed when irreversible neurologic damages have already taken place and on firm clinical suspicion, immediate anti-tuberculosis therapy is recommended, regardless of the results of the tests. Early and reliable diagnosis of TBM still poses a great challenge though delay in diagnosis and treatment is regarded as major contributing factor.

In cases of TBM, the CSF pressure is typically higher than normal, appear clear or slightly turbid. If the CSF is left to stand, a fine clot resembling a pellicle or cobweb may form. This

faintly visible "spider's web clot" is due to the very high level of protein in the CSF, typical of this condition. In proven cases of TBM, hemorrhagic CSF also has been recorded attributing to fibrinoid degeneration of vessels and hemorrhage.

CSF typically has lymphocytic pleocytosis.In adults, the mean WBC count averages around 223 cells/µL (range, 0-4000 cells/µL), while the proportion with neutrophilic pleocytosis (>50% neutrophils) averages 27% (range, 15-55%) and the proportion with normal cell count averages 6% (range, 5-15%). In children, these numbers are 200 cells/µL (range, 5-950 cells/µL), 21% (range, 15-30%), and 3% (range, 1-5%), respectively. (Gracia – monco 1999)

Usually, there is shift to lymphocytic predominance over the ensuing 24 to 48 hours (Verdon et al., 1996 as cited in Gracia – monco 1999), although occasionally neutrophils persist, resulting in the so called persistent neutrophilic meningitis (Peacock as cited in Gracia – monco 1999). This syndrome can also occur in HIV infected patients, particularly when meningitis is caused by multidrug resistant mycobacteria (Sánchez-Portocarrero et al 1996., as cited in Gracia – monco 1999). In 20-25 % of HIV negative patients neutrophilic predominance is present (Verdon et al 1996., as cited in Gracia – monco 1999). It has also been described in immunocompromised patients who are not infected with HIV with TBM. (Mizullani et al 1993 as cited in Gracia – monco 1999).

For patients with HIV and/or immunosuppression, while the mean WBC count in the CSF is 230 cells/µL, as many as 16% of HIV-infected patients may have acellular CSF, compared with 3-6% of HIV-negative patients. Patients whose CSF samples are acellular may show pleocytosis if a spinal tap is repeated 24-48 hours later. The proportion who have neutrophilic pleocytosis of the CSF (>50% neutrophils) is 42% (range, 30-55%). (Gracia – monco 1999).

Within a few days after commencement of anti-TB therapy, the initial mononuclear pleocytosis may change briefly in some patients to one of polymorphonuclear predominance, which may be associated with clinical deterioration, coma, or even death. This therapeutic paradox has been regarded by some authors as virtually pathognomonic of TBM. (Smith1975 as cited in Gracia – monco 1999). This syndrome is probably the result of an uncommon hypersensitivity reaction to the massive release of tuberculoproteins into the subarachnoid space. (O Toole et al., 1969 and Udani et al., 1971 as cited in Gracia – monco 1999).

CSF cell counts and cell morphology by cytospin studies form an important preliminary step in TBM diagnosis.Identifying the atypical polymorphonuclear predominance and at times acellular forms of TBM is essential. The appearance of reactive monocytes and floating tubercles, which are clumps of reactive monocytes with mononuclear cells such as small lymphocytes and polymorphonuclear cells, are diagnostic of TBM. Giant cells are multinucleated inflammatory cells and are observed in 5 % and floating tubercles in 23 % of cases of TBM by routine CSF cytospin studies, as per the experience of studying a large number of CSF samples of TBM cases.

The proportion with depressed glucose levels (< 45 mg/dL or 40% of serum glucose) averages 72% (range, 50-85%) for adults and 77% (range, 65-85%) for children. (Gracia – monco 1999)

CSF typically has an elevated protein level, and marked hypoglycorrhachia. The mean protein level in adults averages 224 mg/dL (range, 20-1000 mg/dL), and in children it is 219

mg/dL (range, 50-1300 mg/dL). The proportion with a normal protein content averages 6% (range, 0-15%) for adults and 16% (range, 10-30%) for children.

While HIV-infected patients generally have a mean protein level of 125 mg/dL (range, 50-200 mg/dL), as many as 43% of the patients may have a normal CSF protein content. The proportion who have depressed CSF glucose levels (< 45 mg/dL or 40% of serum glucose) averages around 69% (range, 50-85%).

If tuberculosis is suspected, the presence of normal protein and glucose levels, and even the absence of white cells in the CSF, should not be considered a reason for not processing with a search for tubercle bacilli both on stained CSF specimens and in culture. (Gracia – monco 1999)

Direct Ziehl-Neelsen (ZN) staining of the cerebrospinal fluid for acid-fast bacilli remains the cornerstone of rapid diagnosis, but this technique lacks sensitivity, but requires large volumes of CSF, and meticulous microscopy to achieve the best results. One of the underlying difficulties is due to the fact that tubercle bacilli are paucibacillary and are shed intermittently in the CSF.It has been recommended that collection of three to four serial samples and spinning of large volumes of CSF for 30 minutes may enhance the rate of detection of AFB in smear microscopy. However, repeat collection of such large volume of CSF is practically impossible. Though less sensitive, smear microscopy is a simple, rapid, cost effective adjunct for the diagnosis of TBM.

A positive smear result is present in an average of 25% (range, 5-85%) of adults and only 3% (range, 0-6%) of children, whereas the numbers with a positive CSF culture average 61% (range, 40-85%) and 58% (range, 35-85%) for adults and children, respectively. (Gracia – monco 1999).

Cultures of CSF by the conventional method using Lowenstein Jenseen(LJ) medium can take 4 to 8 weeks. Automated AFB culture system provides a highly sensitive and rapid tool for the isolation and drug susceptibility testing of MTB, from CSF of TBM patients. Use of a solid medium in conjunction with the automated systems like BACTEC 12B medium is essential for optimal recovery of MTB from CSF specimens. (Venkataswamy et al., 2007 and Jalesh et al., 2010). Drug-resistant tuberculosis is an increasing problem worldwide. MDR TBM was observed in 2.4% of cases (Nagarathna et al., 2008) Several PCR-based tests are also designed for the rapid detection of MDR TB Strains.

Among HIV, on an average the number of positive CSF culture is 23%.

CSF culture for mycobacteria (Gracia – monco 1999) must be processed in a biological safety cabinet.

Indirect modes of diagnosing TBM are by the use of immunodiagnostic methods. These are alternative and adjunctive tests and not confirmatory for TBM diagnosis. These methods have been evaluated, but there are variable immune responses in TBM patients at different stages of the infection posing a barrier to the detection of mycobacterial antibodies in CSF samples. The patients at the chronic stages of TBM have a myriad of antibody responses to all major antigens of MTB, while patients at the early stages have lower detectable antibody response. In addition, interpretation of mycobacterial antibodies in the CSF must take into account the contribution of antibodies from the plasma for correlative interpretation.

Genomic analysis and antigen mining of MTB have yielded novel, more specific antigens, such as early secretory antigenic target 6 (ESAT-6), 38-kDa antigen, 11kda. One approach to

provide direct evidence of existing infection is the detection of the presence of specific antigens in the circulating CSF. The immunological tests especially the ELISA can be used to detect MTB antigens such as Lipoarabinomannan (LAM) 38 kDa antigen, A60 antigen, antigen 5 and 6 by use of epitope specific monoclonal antibodies. Nevertheless, the diagnostic efficiency of the antigen-based approach is still unsatisfactory.

The antibody detection ELISA are the more popular test utilized in immunodiagnosis. These can be utilized to detect either the total antimycobacterial Antibodies using the MTSE (MTB soluble extract) or specific antibody to defined antigens such as LAM, 38 kDa, A 60, 30kDa 1,6kDa. There are several secretory MTB antigens defined to detect humoral immune responses: some of these are early secretory antigenic target 6 (ESAT-6), ag85 complex, MPT63, MPT 64, MPT 70, 14 kDa, 19KDa, 38 kDa, 85B, 45/47, ORF 9, ORf 10 and culture filtrate (CF) antigens. The overall percentage of TBM diagnosis, by immune diagnosis, does not exceed 70 to 87 % (Akepati 1989, Chandramuki 1989, Akepati et al., 2002) Detection of mycobacterial immune complexes (IgG and IgM) also aid in the diagnosis of large proportion of TBM cases (Patil et al., 1996).

A number of strategies have been attempted to improve the laboratory diagnosis of TBM. Nucleic acid amplification tests (NAAT) show potential roles in confirming the diagnosis of TBM.

PCR is the most widely applied alternative rapid diagnostic technique for TBM. A prospective large scale study to evaluate the efficacy of an in-house developed IS6110 uniplex PCR in the diagnosis of clinically suspected of TBM showed all culture-positive samples were positive by the PCR assay. The assay was found to be positive in 70% of the samples with a clinical diagnosis of TBM with an observed sensitivity of 76.37% (negative predictive value 59.90%) and a specificity of 89.18% (positive predictive value 94.69%). A diagnostic accuracy of 80% was seen in patients with a clinical diagnosis of TBM. Patients with a clinical diagnosis of TBM were found to be 9.38 times more likely to be PCR-positive (Rafi et al., 2007).

MTB belongs to the group of intracellular bacteria, which replicate within resting macrophages. During the early stages of the CNS infection, the tubercle bacilli in the CSF are immediately phagocytosed by the macrophages, leading to the scarcity of mycobacterial markers in the circulating CSF. Thus, no such tests have yet become available for early diagnosis of active TBM with the requisite sensitivity and specificity.

Use of neurochemical markers has been investigated in patients with TBM wherein decreased levels of taurine and vitamin B-12 and increased levels of phenylalanine were noted in patients with TBM. Levels of nitrite, its precursor arginine and homocysteine were significantly higher in patients with TBM.

Adenosine deaminase activity measurement could be an inexpensive, valuable tool in the diagnosis of early TBM. (Janvier et al., 2010). Despite its many limitations, tuberculin skin test an indirect test to demonstrate the cell mediated immune response in the body to mycobacterial antigens which by necessity, remains in widespread use. The Centers for Disease Control and Prevention, the American Thoracic Society, and the Infectious Disease Society of America have updated the guidelines, and they are quite useful in practice. Cutoff points for induration (5, 10, or 15 mm) for determining a positive test result vary based on the pretest category into which the patient falls. Negative results from the purified protein

derivative test do not rule out tuberculosis, if the 5-tuberculin test skin test result is negative, repeat the test with 250-tuberculin test. Note that this test is often nonreactive in persons with TBM.

A combination of direct microscopy on ZN staining, culture by conventional LJ media and automated culture system in clinically suspected cases, supported by other laboratory parameters increases the sensitivity of diagnosing TBM as compared to any single method.

TBM still remains a diagnostic challenge because of inconsistent clinical presentation and lack of rapid, sensitive and specific tests. The ideal diagnostic test for TBM should combine acceptable sensitivity and specificity with speed, low cost and ease of execution in a clinical laboratory in developing countries where the disease is more prevalent.Finally the success of TBM diagnosis depends on continued dialogue and collaboration between attending physician and/or laboratarian, medical microbiologist, immunologist, chemical analyst, molecular biologist and pathologist.

Failure to respond to treatment should prompt a search for fungal infections or malignancy.

6. Fungal meningitis

Fungal meningitis is rare, but can be life threatening. The most common cause of fungal meningitis among people with immune system deficiencies, like HIV, is Cryptococcus, Candida, which can lead to meningitis in rare cases, especially in pre-mature babies with very low birth weight. People with immunodeficiencies are at a higher risk for histoplasma meningitis. Histoplasma is found primarily in soil or bird/bat droppings in the Midwestern United States, although it can be seen in other places. Soil in Southwestern United States and northern Mexico contain the fungus Coccidioides, which can cause fungal meningitis. People at higher risk include African Americans, Filipinos, pregnant women in the third trimester, and immunocompromised persons.

Lumbar puncture is also part of the routine evaluation. CSF is tested for opening pressure WBC and differential, glucose, protein, culture, antibodies/antigens, India ink stain (Cryptococcus). However, repeated sampling is often required because diagnosis of non-HIV-associated cryptococcal meningitis, coccidioidal meningitis, histoplasmosis, and candidal meningitis can be difficult.

Up to three sets of blood cultures should be taken in all patients; they may be positive when candidal, histoplasmal, or cryptococcal meningitis is associated with disseminated disease.

CSF analysis usually reveals lymphocytic pleocytosis with raised protein and low sugar levels. In our study, 52% of the patients had < 20 cells/cmm while only 20% had > 100 cells/cmm. The diagnosis of cryptococcal meningitis can be established with India ink stain in > 50% of the cases of cryptococcal meningitis in HIV-negative cases and in > 90% of patients with AIDS (Satishchandra et al., 2007).

The India ink or Nigrosin should be shaken well before every wet mount preparation. Too much stain makes the background too dark and the stain should be regularly checked for quality control, contamination by examining just the stain under a microscope. It will be positive when about 10^3 -10^4 colony-forming units (CFU)/ml are present in a CSF sample.

AIDS patients have larger concentrations of yeast ranging between 10^5 -10^7 CFU/ml (Satishchandra et al., 2007)

False positive readings may occur with air bubbles, myelin globules or RBC and leukocytes. Air bubbles under high power, will be hollow and will not show the typical cell with characteristic nuclei. Monocytes and neutrophils have a crenated margin (and not the entire margin as is seen in cryptococcal cell) and will not show the characteristic refractive cell inclusions, and the luminous halo around the cell is not well demarcated like in Cryptococcus. Centrifuging the CSF to 500 rpm for about 10 minutes and performing India ink stain on the pellet can improve the sensitivity of this test. (Casadevall and Perfect.1998, as cited in Satishchandra et al., 2007)

The CSF sample should also be evaluated for cryptococcal antigen assay that is positive in almost all cases except very early in the disease or in those with very high titers due to prozone effect and in certain patients with cryptococcomas. (Brew BJ 2001, as cited in Satishchandra et al., 2007)

The methods used for antigen detection are latex agglutination test and enzyme immunoassay and are > 90% sensitive and specific. Cryptococcal antigen titers usually decrease with treatment, but it can remain at low titers for long periods even after effective therapy. (Lu, 2005 as cited in Satishchandra et al., 2007)

Antigen testing should go hand in hand with culture, since low levels of antigen titre persist for a long time even after clearance of the organism from CSF. Occasional false positive reaction is also encountered, particularly in the presence of rheumatoid factor or infection with the yeast Trichosporon spp. Pronase treatment is necessary when serum, instead of CSF, is to be tested.

A positive fungal culture is the gold standard for diagnosis of Cryptococcal infection and CSF samples show fungal growth in almost all the cases. In our series, fungal cultures grew C. neoformans in 100% of the cases. Fungal cultures also help to determine the species of the infecting organism and sensitivities to various antifungal agents. Globally, all cases of Cryptococcosis in AIDS patients are due to var grubii, followed by var neoformans. Our observations highlight the fact that the rate of C.gattii in this part of the country is comparatively low (2.8%) (Nagarathna et al., 2010). Drug susceptibility testing of the fungal isolates is not routinely done except in cases of recurrent disease. (Satishchandra et al., 2007) and has become important due to emerging antifungal resistant fungi causing infections in patients with AIDS. However, in a pilot study conducted by us did not reveal resistance to any of the routine antifungal drugs.

The routine processing of CSF for chronic meningitis and subacute meningitis cases for fungal culture is a good surveillance procedure so as not to miss any Cryptococcal, Candidial and occasionally the Cladosporial CNS infections This is more essential because of the incidence of HIV. In disseminated Cryptococcal infections, additional sampling of sputum, urine and blood is useful. The fungus often isolated is Cryptococcus. The other CNS fungal agents are almost never isolated except rarely the Cladosporium. Cytospin studies can identify the yeasts when India ink preparation is negative.

Pan-fungal PCR has been a promising aid in rapid, early diagnosis of invasive fungal infection (IFI).On the other hand, it has the potential to detect all fungal species.

Epidemiological studies now indicate that the spectrum of fungal pathogens has expanded well beyond Aspergillus fumigatus and Candida species (Pfaller and Diekema. 2004. as cited in Lau et al., 2007). However, current culture-based phenotypic methods are insensitive and slow, may initially be nonspecific, and require considerable expertise for correct morphological identification of less common or unusual fungi (Alexander, and Pfaller. 2006, Chen et al., 2002 as cited in Lau et al., 2007). Additional drawbacks of conventional culture include the failure of zygomycetes to grow

- when hyphal cells have been damaged during processing (Larone, 2002. as cited in Lau et al., 2007)
- or the collection of tissue biopsy specimens directly into formalin fixative for paraffin embedding when IFIs are not suspected clinically (Iwen 2002 as cited in Lau et al., 2007)
- or when limited material is available.

Recent efforts to improve the sensitivity and specificity of diagnostic tests have focused on culture-independent methods, in particular nucleic acid-based methods, such as PCR assays. These can be applied to fresh and formalin-fixed, paraffin-embedded sections. Numerous studies have highlighted the advantages of using PCR technology to detect viable and nonviable fungal pathogens in a variety of clinical specimens. The majority of assays target multicopy genes, in particular the ribosomal DNA (rDNA) genes (18S, 28S, and 5.8 S) and the intervening internal transcribed spacer (ITS) regions (ITS1 and ITS2), in order to maximize sensitivity and specificity.

To date, most assays have been designed to detect Candida or Aspergillus species only. Given that more than 200 fungal species have been reported to cause disease in humans and companion animals, the clinical utility of a species-specific or even a genus-specific assay is limited. Sequence-based identification of PCR products is a sensitive alternative, provided that accurate sequences have been submitted to public databases, e.g., GenBank. A panfungal PCR assay targeting the internal transcribed spacer 1 (ITS1) region of the ribosomal DNA gene cluster successfully detected and identified the fungal pathogen in 93.6% and 64.3% of culture-proven and solely histologically proven cases of IFI, respectively. A diverse range of fungal genera were identified, including species of Candida, Cryptococcus, Trichosporon, Aspergillus, Fusarium, Scedosporium, Exophiala, Exserohilum, Apophysomyces, Actinomucor, and Rhizopus. The results support the use of the panfungal PCR assay in combination with conventional laboratory tests for accurate identification of fungi in tissue specimens. (Lau et al., 2007).

Galactomannan(GM) is a component of the cell wall of the mould Aspergillus and is released during growth. Detection of GM in blood is used to diagnose invasive aspergillosis infections in humans. Eg Platelia galactomannan enzyme immunoassay (Bio-Rad). Although the test is approved by the FDA for use with patients with neutropenia and those undergoing stem cell transplantation, controversy about the test's utilization exists. Although initial results were promising, various sensitivities and specificities (29 to 99%) have been reported recently in prospective studies (Zedek and Miller 2006). The Aspergillus GM test was performed on CSF and serum. Detection of Aspergillus GM in CSF may be diagnostic of cerebral aspergillosis. It is suggested that the Aspergillus CSF GM index might be diagnostic for cerebral aspergillosis in patients at high risk for aspergillosis and with a compatible neurological disease. (Claudio Viscoli et al., 2002).

A positive result supports a diagnosis of invasive aspergillosis (IA) and should be considered in correlation with clinical condition, microbiologic culture, histological examination of biopsy specimens, and radiographic evidence, and other laboratory parameters.

A negative result does not rule out the diagnosis of IA.When there is a strong suspicion of IA, repeat testing is recommended. Patients at risk of IA should have a baseline serum tested and should be monitored twice a week for increasing GM antigen levels. GM antigen levels may be useful in the assessment of therapeutic response. Antigen levels decline in response to antimicrobial therapy. False-positive results are reported to occur at rates of 8% to 14%.

The Glucatell (1r3)-β-D-glucan (BG) detection assay was studied as a diagnostic adjunct for IFIs and a serum BG level of 60 pg/mL was chosen as the cutoff. IFIs included candidiasis, fusariosis, trichosporonosis, and aspergillosis. Absence of a positive BG finding had a 100% negative predictive value, and the specificity of the test was 90% for a single positive test result and 96% for 2 sequential positive results. The Glucatell serum BG detection assay is highly sensitive and specific as a diagnostic adjunct for IFI.

Glucatell assay may be a useful diagnostic adjunct for the diagnosis of invasive fungal infection, particularly in high-risk populations. The positivity of this test, particularly when used in a serial fashion, often precedes the microbiological or clinical diagnosis of invasive fungal infection. This cell wall component has the advantage of being present and detectable in a variety of fungal infections. **(**Zekaver etal., 2004).

7. Cysticercal meningitis

At NIMHANS, at the department of Neuromicrobiology, the neurocysticercal immunology has revealed anticysticercal antibody in CSF of approximately 5-10 % of cases, involves the use of whole cyst antigen often called porcine cyst sonicated, partially purified antigens such as antigen B, defined antigens such as 8-10 kDa, 23kDa, 64-68 kDa by EITB, antigens secreted while cysts are maintained invitro, namely excretory /secretory (E/S) antigens. Qualitative ELISA kit for the detection of anticysticercal antibodies in CSF, which uses purified E/S antigens of Cysticercus cellulosae maintained *in vitro,* was developed by the department of Neuromicrobiology in collaboration with Astra Research laboratory/organization E/S antigens of Cysticercus cellulosae for use in immunodiagnosis and vaccine preparation (CYSTI-CheX) *European patent WO/90/08958, 1990-dtd 9/7/1991*.The inclusion of anticysticercal antibody especially applied to CSF in endemic countries should form a routine laboratory test, as Neurocysticercosis can have protean clinical manifestations.

8. Carcinomatous meningitis

Leptomeningeal carcinomatosis occurs in approximately 5% of patients with cancer. The shedding of atypical and malignant cells into the subarachnoid space can itself trigger meningitic inflammation which is influenced by the inflammatory cells such as polymorphonuclear cells and mononuclear cells along with atypical CSF cells. The definitive identification of the tumour cells in CSF can provide useful information about the cause of chronic meningitis and nature of the malignant meningitis whether primary or secondary metastasis.

The best mode available is the cytological examination of CSF by cytospin in all cases of chronic meningitis. Atypical or definitive malignant cells in the CSF can arise from primary

CNS tumours such as ependymoma, choroids plexus papilloma, pinealoma, medulloblastoma, glioblastoma multiforme and many other tumors or secondary metastasis in the CNS, originating from bronchopulmonary, breast, or ovarian malignancies.

- Tests to aid in the diagnosis of TBM-
- CSF cell analysis using cell counting chamber and cytospin, acid fast smear examination of cytospin smear, either by Kinyouns or Ziehl Neelsen stain, or auramine O staining, CSF culture by conventional methods on LJ slopes or Middlebrook liquid media, and or by using automated systems. Detection of Mycobacterial DNA by polymerase chain reaction, adjunctive add on tests by immunological modes to detect mycobacterial antigens, antimycobacterial antibodies / mycobacterial immune complexes.Multimodal approach is essential in the diagnosis of TBM.
- Tests to detect anticysterical antibodies in CSF and / serum samples.
- Serum /CSF VDRL and Treponema pallidum particle agglutination test tests to rule out or establish Neurosyphilis.
- CSF cytospin to detect atypical / malignant cells to aid in diagnosis of secondary or primary CNS neoplasia.
- Mycological exercises on CSF 1 by India ink, Cryptococcal antigen tests and culture to establish Cryptococcal aetiology
- CSF C- reactive protein assay to establish chronic microbial aetiology especially partially treated pyogenic meningitis.
- Additional tests to rule out /establish neurobrucellosis, neuroborreliosis, neuroaids.
- Wetmout preparation to detect motile acanthamoeba species in CSF t establish cases of primary amoebic granulomatous meningitis.
- Meningeal biopsy and histology, only in exceptional cases of idiopathic chronic meningitis.Microarrays, or biochips, are a new technology that can allow rapid detection of bacterial genetic materials. The microarray method provides a more accurate and rapid diagnostic tool for bacterial meningitis compared to traditional culture methods. Clinical application of this new technique may reduce the potential risk of delay in treatment. (Ben et al., 2008)

Inadequacy of current diagnostic tools for bacterial meningitis is typical of less-developed countries. Properly interpreted test is a key tool in the diagnosis of a variety of diseases. Proper evaluation of CSF depends on knowing which tests to order, normal ranges for the patient's age, and the test's limitations. Diagnostic uncertainty can be decreased by using accepted corrective formulae. (carbonnelle.E). Although improvement in diagnosis is urgently required, the emphasis should be on identification of causes so that appropriate interventions can be applied. (GE Enwere)

9. Quality assurance (QA)

Follow manufacturer's instructions for media and diagnostic kits.
Follow standard QA procedures for laboratory equipment and test methods

10. Biosafety

- Perform all work on suspected isolates in a safety cabinet.
- Wash hands particularly after collection and handling of the clinical specimen.
- Use leak proof containers in sealed plastic bags for transportation of the sample.

11. Referral and notification

Isolates associated with clustering, outbreaks, diagnostic dilemma, epidemiological importance, with unusual or unexpected resistance patterns have to be notified/referred, for confirmation, strain characterization antimicrobial susceptibility testing, serotyping etc.

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Dr P Satishchandra Director and Vice-Chancellor, Professor of Neurology NIMHANS Bengaluru Karnataka, India.

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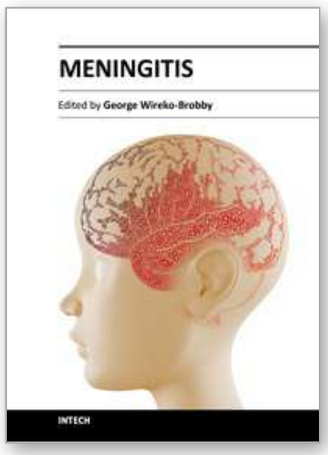

Meningitis

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Meningitis is a medical emergency requiring a rapid diagnosis and an immediate transfer to an institution supplied with appropriate antibiotic and supportive measures. This book aims to provide general practitioners, paediatricians, and specialist physicians with an essential text written in an accessible language, and also to highlight the differences in pathogenesis and causative agents of meningitis in the developed and the developing world.

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